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Natural abundance of stable nitrogen isotopes reflect changes in pelagic food webs and mobility of size classes of the north Iberian sardine (*Sardina pilchardus*).

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The natural abundance of stable nitrogen isotopes ($\delta^{15}N$) in sardine (*Sardina pilchardus*) and plankton was determined from samples collected between 1998 and 2003 in the northwestern shelf of the Iberian Peninsula with the purpose of studying the variability in the trophic position of sardine. Sardines were grouped in three size-length classes (small: < 16 cm; medium: 16-20 cm; large: > 20 cm). Plankton samples were size-fractionated between ca. 0.7 and 2000 μm of individual length. The plankton at the base of the pelagic food web in the western shelf region (Galicia) was more enriched in $\delta^{15}N$ than plankton in the northern and eastern region (Mar Cantábrico), likely because of upwelling. Variability in $\delta^{15}N$ in sardine was related both to changes in feeding strategies during growth and to the mobility of size classes between adjacent shelf regions. Large sardines showed regional differences in $\delta^{15}N$ similar to those of small plankton, suggesting that old specimens remained for long periods in the same region. In contrast, small and medium-sized sardines have similar $\delta^{15}N$ values in all regions. We hypothesize that interannual differences in $\delta^{15}N$ of sardine are caused mainly by variations in the mobility of large size classes, as the isotopic signature of the small sardines was relatively less variable and suggests low variability in the plankton food web. In turn, such variations would be related to changes in the size of the sardine population in the north Iberian shelf.

Keywords: sardine, plankton, feeding, stable isotopes, migration, NW Iberian Peninsula

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Introduction

The natural abundance of stable isotopes was used as a tracer of fish migrations between areas containing different food sources (e.g. Hanson et al., 1997; Doucett et al., 1999; Satterfield and Finney, 2002). Its applicability depends on the existence of a clear isotopic signature at the base of the food web in each of the feeding areas exploited by the target fish species. Migrating fish will integrate the isotopic composition of its diet and therefore their values of natural isotopic abundance will be different from those of fish feeding only in the same area.

Two different types of food webs are expected in the shelf ecosystem in the northwestern Iberian Peninsula. On the one hand, the seasonal upwelling from March to October produces significant inputs of nutrients that sustain high values of plankton biomass (Tenore et al., 1995; Alvarez-Salgado et al., 2002). The upwelling was forced by northern winds that produce the Ekman transport of surface waters offshore. The effects of upwelling in the western shelf (Galicia) were higher than those in the northern region (Mar Cantábrico), which received lower nutrient inputs (Botas et al., 1990). Furthermore, the interaction of upwelling waters with the coastal morphology of Galicia, especially near the large coastal inlets or rias, further enhances plankton biomass through the export of organic matter from the rias to open shelf waters (Alvarez-Salgado et al., 2000). During upwelling, a metazoan food web based on new nutrients from the upwelling (as nitrate) would be expected (Cushing, 1989). On the other hand, a coastal poleward current flows during autumn and winter in an opposite direction to upwelling surface currents (Frouin et al., 1990). Southern winds contribute to the progress of the poleward flow along the shelf (Cabanas, 1999; Alvarez-Salgado et al., 2003) from Galicia to the Mar Cantábrico (Bode et al., 2002). Waters inside the poleward current contain less nutrients than waters fertilised by the upwelling (Bode et al., 2002; Alvarez-Salgado et al., 2003) and display low plankton biomass (Fernández et al., 1991; 1993). In this situation, a microbial food web based on the remineralisation of the organic matter would be expected. As light isotopes are preferentially mobilised in metabolic processes, plankton growing on nutrients remineralised in situ is expected to have a lower proportion of heavy isotopes than plankton growing on new nutrients originated in deep waters, as those supplied by upwelling (e.g. Wada and Hattori, 1991).

Large populations of sardine exploit plankton biomass along the Atlantic Iberian shelf. Previous studies showed the existence of an historical minimum in both commercial fishery landings and acoustic estimations of sardine biomass between 1998 and 2000, particularly in Galicia (ICES, 2003). The reduction in sardine stocks until 1999 was explained because of the depletion of the stocks in coincidence with a regime shift in oceanographic conditions (Carrera and Porteiro, 2003). Upwelling intensity was shown to affect in different ways, both positive and negative (Dickson et al., 1988; Roy et al., 1995), sardine recruitment in this area but the main direct effect was the transport of eggs and larvae offshore by northern winds (Guisande et al., 2001). In this way, strong upwelling during the recruitment season would decrease the probability of survival of sardine larvae as they are dispersed to outer shelf and oceanic zones. In contrast, southern winds favour the progress of the poleward current, which tend to accumulate fish larvae near the coast where plankton biomass and production are high (Fernández et al., 1993). At high population sizes, sardines spawning and distribution areas extend over the whole continental shelf and the adults display feeding migrations to the upwelling area off Galicia, while at low population sizes a reduction in the mobility of adult sardines between the Mar Cantábrico and Galicia was expected (Carrera and Porteiro, 2003). In the latter case, sardines will remain in the same areas where they were hatched. Previous studies showed the relationships between stable isotope composition of plankton and sardines in this region, which feed predominatly on zooplankton preys (Bode et al., 2003; 2004).

The present study analyse the variability in the trophic position of sardine, estimated from the natural abundance of ^{15}N, in relation to variations in plankton along the north-

western shelf of the Iberian Peninsula. The objective is to determine the possible of permanence of sardines in some shelf areas during certain years, related to variations in the size of stocks. Three hypothesis are tested: (1) plankton from areas subject to intense upwelling will be enriched in ^{15}N relative to weak or non-upwelling areas; (2) the sardines feeding in upwelling areas will acquire the isotopic signature of plankton, resulting more enriched in ^{15}N than sardines feeding in non-upwelling areas, and (3) the differences in ^{15}N between sardines collected in different areas will be maximal when the stocks are reduced.

Material and methods

Samples were collected during PELACUS cruises, between March – April, from 1998 to 2003 (Fig. 1, Table 1). Sardine samples were collected by means of a pelagic trawl with vertical aperture of 24 m for 15-30 min of effective sampling. Each specimen was measured (± 5 mm), weighed (± 0.2 g) and dissected to obtain portions of white muscle and stomach contents, which were stored frozen for isotopic determinations. As previous studies showed a decrease of natural abundance of ^{15}N with length (Bode et al., 2003; 2004), sardines were grouped in three size-length classes (small: <16 cm; medium: 16-20 cm; large: >20 cm). Plankton samples were collected during the night using a conical net of 20 μm mesh-size from 100 m depth to the surface at stations distributed over the shelf up to the shelf break. Samples were subsequently fractionated through sieves of 40, 80, 200, 500, 1000 and 2000 μm and each fraction was carefully washed with filtered seawater, transferred to glass-fibre filters and stored frozen. The plankton retained by the 2000 μm sieve (generally large salps) was not used in this study. In addition, water from the surface was prefiltered through a sieve of 20 μm mesh-size and subsequently filtered through Whatman glass-fibre GF/F filters to characterise seston < 20 μm. These filters were stored frozen and processed as the filters with net plankton samples.

Natural abundance of ^{15}N ($\delta^{15}N$) in plankton and sardine was measured in a isotope-ratio mass spectrometer (Finnigan Matt Delta Plus) coupled to an elemental analyser (Carlo Erba CHNSO 1108) after oven drying plankton (50 °C, 24 h) or freeze-drying sardine samples. The determinations were calibrated against atmospheric nitrogen. Precision (± 1 s.e.) of triplicate $\delta^{15}N$ determinations was better than 0.03 ‰ (Bode et al., 2003; 2004; Bode and Alvarez-Ossorio, 2004).

Estimations of the size of sardine populations were taken from the acoustic measurements collected during the PELACUS cruises (ICES, 2003). For the purpose of the analysis of spatial variability, the ICES subdivisions indicated in Fig. 1 were used.

Environmental variability between years was exemplified by the poleward index (Cabanas, 1999), an estimate of the northward transport of water along the northwestern shelf of the Iberian Peninsula computed at 43° N, 11° W from geostrophic winds. The values of the poleward index are proportional to the intensity of the poleward current that flows near the shelf break during autumn and winter (Alvarez-Salgado et al., 2003). In this paper, the values of the index (in km month^{-1}) are the average of the transport between October and December of the preceding year.

Results and discussion

Spatial variations

Due to the insufficient number of samples in the different combinations of cruises and zones (Table 1), a full factorial analysis of variability between areas and years was not possible. However, in the case of plankton, there were significant differences between size-classes, particularly between plankton 500 and 500 - 2000 μm (Fig. 2 A). An ANOVA considering $\delta^{15}N$ values grouping these plankton classes in the two main subregions, Galicia and Mar Cantábrico (Table 2), showed significant differences between classes ($p < 0.05$) and moreover between subregions ($p < 0.001$). The latter were consistent for both plankton classes considered, as there was no significant interaction between the factors size-class and zone. The subsequent analysis of plankton (all size-classes combined) by all sampling zones showed that samples from S Galicia were significantly enriched in $\delta^{15}N$ relative to samples in other zones (Fig. 2 B). Therefore, plankton in S Galicia must have grown on dissolved nitrogen sources richer in ^{15}N than those found in N Galicia or in the Mar Cantábrico, thus supporting the predictions of our first hypothesis.

Sardine $\delta^{15}N$ varied significantly among size-classes (Fig. 3 A, B), being higher in small individuals than in large ones. Furthermore, the average value of $\delta^{15}N$ in sardines < 16 cm was similar in all zones considered, while those for larger individuals varied depending on the zone. This caused that, considering all sizes together, there was no significant effect of the sampling zone on $\delta^{15}N$, but there was a significant interaction between size-class and zone factors (Table 3). The high isotopic enrichment in young sardines relative to adults was described previously (Bode et al., 2003) and related to the increasing importance of filter feeding with growth in length. In this way, during their first year of life sardines appear to feed almost exclusively on zooplankton, while older individuals include an increasing proportion of phytoplankton in their diet. The inclusion of preys with low $\delta^{15}N$, as phytoplankton has a lower $\delta^{15}N$ than zooplankton, translates in a decrease in the $\delta^{15}N$ of the sardines (Bode et al., 2004). The spatial differences in $\delta^{15}N$ of 16 cm sardines were displayed at various levels of integration, but particularly between S and N Galicia zones (Fig. 3 B).

Since large sardines showed spatial differences in $\delta^{15}N$ similar to those of small plankton, we can assume that sardines have been feeding for relatively long periods in the same zones to acquire the isotopic signature of plankton, as predicted by our second hypothesis. There is no information available on the turnover time of isotopes in sardine muscle, but data for other fish species indicates that it may be in the order of several weeks (Tieszen et al., 1983). Therefore, our data support the hypothesis of a restricted mobility of large sardines between S Galicia and the other zones. In contrast, small and medium-sized sardines have similar average $\delta^{15}N$ values in all zones, which can be explained either as the consequence of a larger mobility of small compared to large sardines or as the preferential feeding on zooplankton by small sardines in all zones. The former explanation seems less probable, as the swimming capabilities are directly related to the size of sardines (Blaxter and Hunter, 1982), while the second agrees with previous findings (Bode et al., 2003; 2004). Nevertheless, the inference of no migration

of adult sardines is based on the assumption that plankton in S Galicia is always enriched in ^{15}N relative to other areas, while studies in N Galicia, for instance, showed significant seasonal variations in $\delta^{15}N$ of mesozooplankton (> 200 μm) related to changes in dissolved inorganic nitrogen and plankton species succession (Bode and Alvarez-Ossorio, 2004). Such time variability in planktonic $\delta^{15}N$, along with the determination of the turnover time of nitrogen isotopes in the muscle of sardine, must be qauntified in future studies to confirm the suitability of ^{15}N as a tracer of sardine movements along the shelf.

Interannual variations

The differences in planktonic $\delta^{15}N$ between sampling years, as exemplified by the values measured in the 20-200 μm size-class, were significant (Fig. 2 C). Plankton collected in 1999, 2000 and 2002 was less enriched, on average, than plankton collected in 2001 and 2003 (ANOVA and Dunnett-C test, $p < 0.05$). However, there was a significant interaction between the sampling year and zone factors (Table 2) indicating that the differences were dependent of the year of sampling. For instance, plankton from S Galicia generally had higher $\delta^{15}N$ values than plankton from the other zones, except in 1999 and 2003 (Fig. 2 C).

Sardine $\delta^{15}N$ also varied significantly between years (Fig. 3 C). The data indicate two main periods: one for 1998 and 1999, which was characterised by relatively low $\delta^{15}N$ values, and a second period comprising samples for 2001 and 2002 (no sardines from the study area were analysed for $\delta^{15}N$ in 2000 and 2003) and characterised by high $\delta^{15}N$. These differences were significant for all sardine size-classes considered (ANOVA, $p < 0.001$), although maximum differences were found for sardines 16 cm. On average, sardines from the 1998-1999 period were 1.2 ‰ enriched in $\delta^{15}N$ relative to sardines collected in 2001. As in the case of plankton $\delta^{15}N$, the significant interaction between the sampling year and the collection zone indicates that the differences between zones were only significant for certain years (Table 3). For instance, samples of 16 cm sardines from the Mar Cantábrico in 1998 had higher mean $\delta^{15}N$ values than those from Galicia, while the mean values for both subregions were equivalent in 1999 and 2001 (ANOVA, $p < 0.001$).

Considering the samples obtained concurrently, mean differences in $\delta^{15}N$ of plankton and sardines collected in the same zone showed a distinct pattern in 1999 than after this period (Fig. 4). In general, sardine $\delta^{15}N$ decreased with increasing plankton $\delta^{15}N$ but the correlation using all available data pairs was not significant ($r = 0.484$, $n = 9$, $p > 0.05$). The results indicate that the mean value $\delta^{15}N$ of 16 cm sardines was relatively constant for a large range of variability of plankton $\delta^{15}N$ values during 2001 and 2002, but decreased with increasing plankton $\delta^{15}N$ during 1999. In addition, in the Mar Cantábrico zone, plankton samples collected in 2001-2002 period were enriched (and sardine samples depleted) in ^{15}N relative to samples collected in 1999. Although based on a reduced number of observations for some periods and zones, these relationships suggest the existence of a marked change in the exploitation patterns of plankton by adult sardines during the study period. On the one hand, similar differences between $\delta^{15}N$ of plankton and sardines would be expected for all sampling years if sardines

would have remained in the same zone for time periods long enough to acquire the isotopic signature of local plankton. In this case, even if the values of planktonic $\delta^{15}N$ had changed because of temporal differences in nutrient sources, average $\delta^{15}N$ values of plankton and sardines would have been positively correlated. The larger mobility of adults sardines for the 2001-2002 period compared to sardines in 1999 would explain the absence of a correlation between the isotopic signatures of sardines and plankton for this period, as the former may have been feeding on plankton captured in different zones. Similar results were reported for the $\delta^{15}N$ content of migratory fish in relation to different food sources while sedentary species clearly display the isotopic signal of local food (e.g. Hanson et al., 1997). On the other hand, the possibility of a change in feeding habits for adult sardines between 1999 and 2001, implying a preferential consumption of less ^{15}N-enriched plankton (as phytoplankton) in the latter, can not be ruled out. In this way, by taking into account an enrichment of 3.4 ‰ between adjacent trophic levels (Vander Zanden and Rasmussen, 2001), the average $\delta^{15}N$ enrichment between plankton and sardines in 1999 (6.68 ± 0.45 ‰, ± s.e.) would imply a trophic position of 3.0, i.e. a primary carnivore. In turn, the average enrichment for sardines collected in 2001 and 2002 (4.65 ± 0.42) corresponds to a trophic position of 2.4, which suggests omnivorous feeding for this period.

Sardine $\delta^{15}N$, population size and oceanographic regime

Changes in isotopic composition of sardines during the study were coincident with a general increase in the biomass of the sardine population in the northwestern Iberian Peninsula, after the minimum reached in 1998 (Fig. 5 A). In addition, the intensity of the winter poleward current decreased until 2000 but increased thereafter (Fig. 5 B). Such variations support the hypothesis of a shift in the oceanographic regime, which in turn would have affected the distribution and the size of sardine stocks (Carrera and Porteiro, 2001). Previous studies have identified interannual and cyclic changes in the poleward current mostly related to general climatologic changes (Cabanas, 1999; Alvarez-Salgado et al., 2003). As the winds favouring the poleward current are contrary to upwelling, it is likely that the increase in the poleward current after 2000 reduced the effect of upwelling on plankton production. Furthermore, the poleward current transports water with low nutrient concentrations (Bode et al., 2002; Alvarez-Salgado et al., 2003), while the upwelling water was generally nutrient-enriched (Castro et al., 2000). The result was a lower phytoplankton production inside the poleward current relative to that of surrounding waters (Fernández et al., 1991; 1993; Alvarez-Salgado et al., 2003). As most of the production occurring in nutrient-depleted poleward waters depends on nitrogen regenerated in situ, the plankton produced during periods of high poleward flow would have a lower $\delta^{15}N$ than the plankton growing on nitrate inputs by upwelling, because of the preferential mobilisation of light isotopes during remineralisation (Wada and Hattori, 1991). In turn, the isotopic signature of plankton would be transmitted to the sardines, resulting in a decrease of $\delta^{15}N$ in sardines during periods of high poleward flow, as observed in our study.

Besides the temporal changes in nutrient sources affecting $\delta^{15}N$ of plankton, there are also other changes in the pelagic food web related to the regime shift. For instance, a decrease in the upwelling would reduce the populations of copepods, one of the main preys of sardine (Varela et al., 1988; 1990), as they are closely linked to events in the

region (Valdés et al., 1990). This would explain the apparent decrease in the trophic position of sardines after 2000, when the poleward flow increased, because the sardines would have to supplement their diet with alternative preys, less enriched in ^{15}N than copepods from upwelling waters.

Conclusions

Significant changes in $\delta^{15}N$ of plankton and sardines in the northwestern Iberian shelf between 1998 and 2003, along with a regime shift in oceanographic conditions, suggest a reduced mobility of adult sardines between shelf areas during periods of low population size. In addition, it is likely that changes in the composition of plankton drive changes in the diet of sardines, which increase omnivorism during periods of low nutrient inputs to the ecosystem.

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Table 1. Number of samples of plankton and sardine collected in the study area during PELACUS cruises between 1998 and 2003.

				no. of samples	
Cruise	Date	Zone	ICES subarea	sardine	plankton
PELACUS 0398	17 March - 7 April 1998	Galicia S	IXa N	94	----
		Galicia N	VIIIc W	10	----
		Cantábrico W	VIIIc Ew	10	----
		Cantábrico E	VIIIc Ee	10	----
PELACUS 0399	14 - 23 March 1999	Galicia S	IXa N	48	20
		Galicia N	VIIIc W	42	20
		Cantábrico W	VIIIc Ew	2	21
		Cantábrico E	VIIIc Ee	12	----
PELACUS 0300	27 March - 10 April 2000	Galicia S	IXa N	----	18
		Galicia N	VIIIc W	----	20
		Cantábrico W	VIIIc Ew	----	28
		Cantábrico E	VIIIc Ee	----	11
PELACUS 0401	2 - 18 April 2001	Galicia S	IXa N	25	166
		Galicia N	VIIIc W	40	51
		Cantábrico W	VIIIc Ew	14	62
		Cantábrico E	VIIIc Ee	10	105
PELACUS 0302	14 - 30 March 2002	Galicia S	IXa N	3	40
		Galicia N	VIIIc W	2	75
		Cantábrico W	VIIIc Ew	----	40
		Cantábrico E	VIIIc Ee	----	97
PELACUS 0303	18 March - 14 April 2003	Galicia S	IXa N	----	22
		Galicia N	VIIIc W	----	42
		Cantábrico W	VIIIc Ew	----	21
		Cantábrico E	VIIIc Ee	----	42

Table 2. Results of ANOVAs on $\delta^{15}N$ of plankton size-classes (grouped as > 500 and 500 - 2000 μm) by sampling zones (grouped in Galicia and Cantábrico shelf subregions), and $\delta^{15}N$ of > 500 μm plankton by sampling year (1999 to 2003) and zone (Galicia and Cantábrico). SS: sum of squares, MS: mean squares, DF: degrees of freedom, F: MS ratio, p: significance.

ANOVA type	Source of variation	SS	DF	MS	F	p
zone by class	zone	14.61	1	14.61	6.22	0.013
	size-class	232.15	1	232.15	98.89	0.000
	zone * size-class	0.98	1	0.98	0.42	0.519
	error	2105.68	897	2.35		
	total	32386.28	901			
year by zone	year	552.24	4	138.06	94.86	0.000
	zone	0.08	1	0.08	0.05	0.816
	year * zone	20.82	4	5.20	3.58	0.007
	error	893.60	614	1.46		
	total	19797.56	624			

Table 3. Results of ANOVAs on $\delta^{15}N$ of sardine size-classes (< 16, 16-20, > 20 cm) by sampling zones (grouped in Galicia and Cantábrico shelf subregions), and $\delta^{15}N$ of 7 16 cm sardines by sampling year (1998, 1999 and 2001) and zone (Galicia and Cantábrico). SS: sum of squares, MS: mean squares, DF: degrees of freedom, F: MS ratio, p: significance.

ANOVA type	Source of variation	SS	DF	MS	F	p
zone by class	zone	0.14	1	0.14	0.32	0.571
	size-class	22.61	2	11.31	26.63	0.000
	zone * size-class	10.78	2	5.44	12.81	0.000
	error	139.69	329	0.43		
	total	39380.87	335			
year by zone	year	53.54	2	26.77	128.49	0.000
	zone	0.02	1	0.02	0.10	0.756
	year * zone	1.49	2	0.75	3.58	0.030
	error	45.62	219	0.21		
	total	26223.98	225			

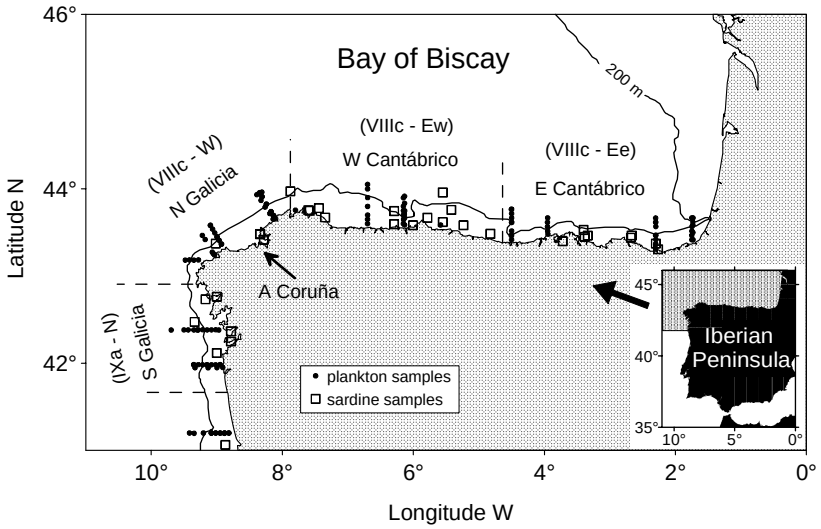

Fig. 1. Map of sampling stations for plankton and sardine. The limits of the zones considered are shown by dashed lines. ICES subareas are indicated in brackets.

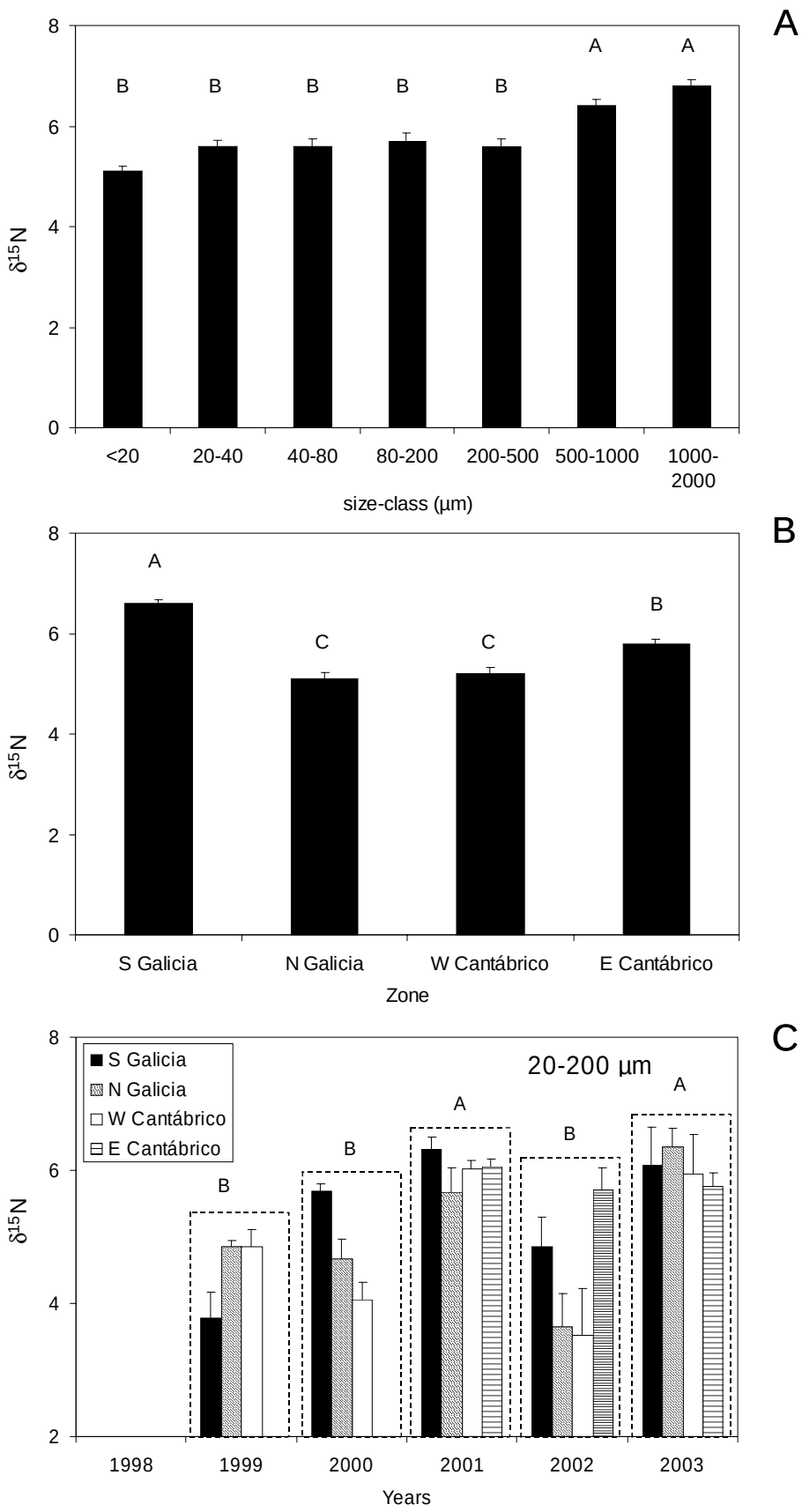

Fig. 2. Mean (+s.e.) $\delta^{15}N$ of plankton for (A) different size-classes (all years and zones combined), (B) shelf zones (all size-classes and years combined) or (C) years by shelf zones (only 20-200 µm size-class). Significant differences between means are indicated by different letters (ANOVA and Dunnett-C test, $p < 0.05$). Only significance between years was analysed in panel C.

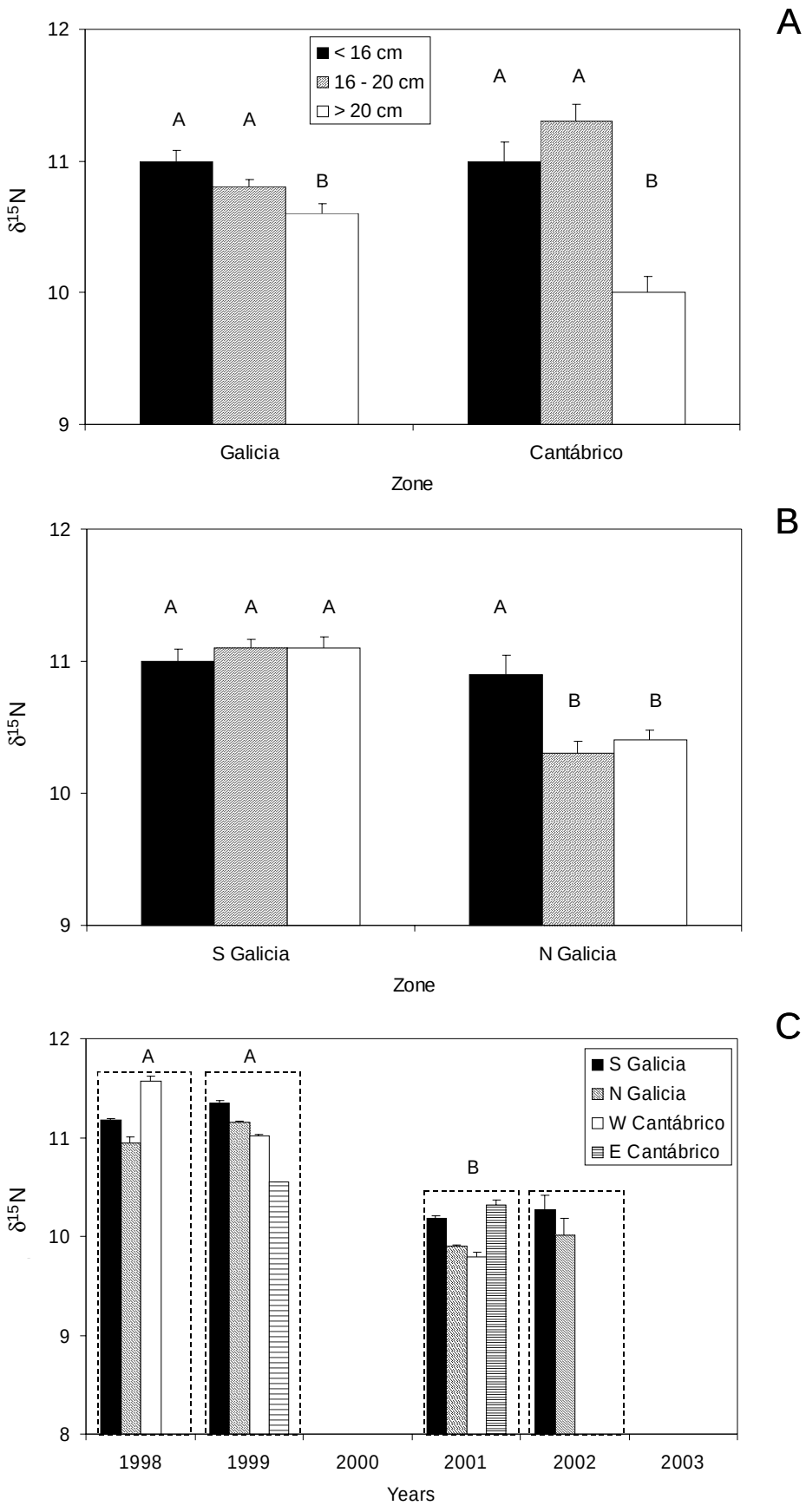

Fig. 3. Mean (+s.e.) $\delta^{15}N$ of sardine size-classes for different shelf zones (A, B) or years by shelf zones (only > 16 cm size-class). Significant differences between means are indicated by different letters (ANOVA and Dunnett-C test, $p < 0.05$). Only significance between years was analysed in panel C.

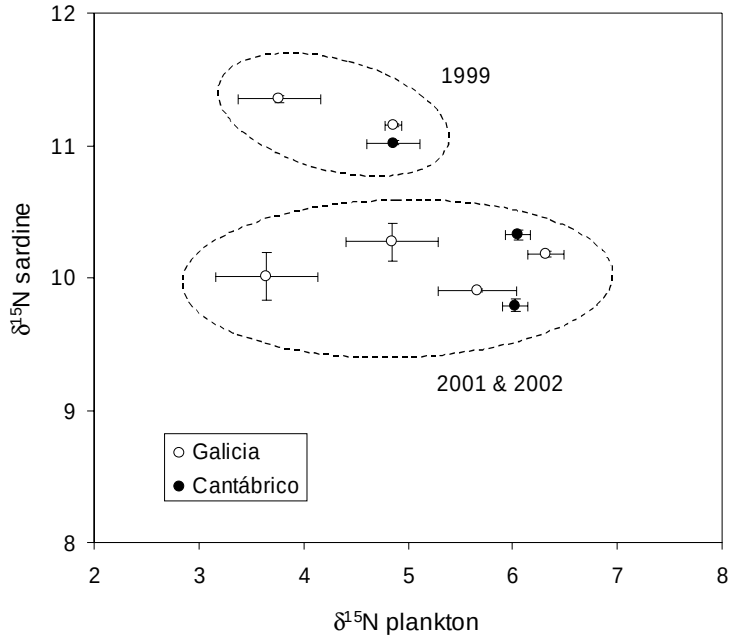

Fig. 4. Plot of mean (± s.e.) $\delta^{15}N$ of 20-200 µm plankton and 7 16 cm sardine concurrently measured in Galicia and Mar Cantábrico zones. The values for 1999 and 2001 - 2002 periods were encirled.

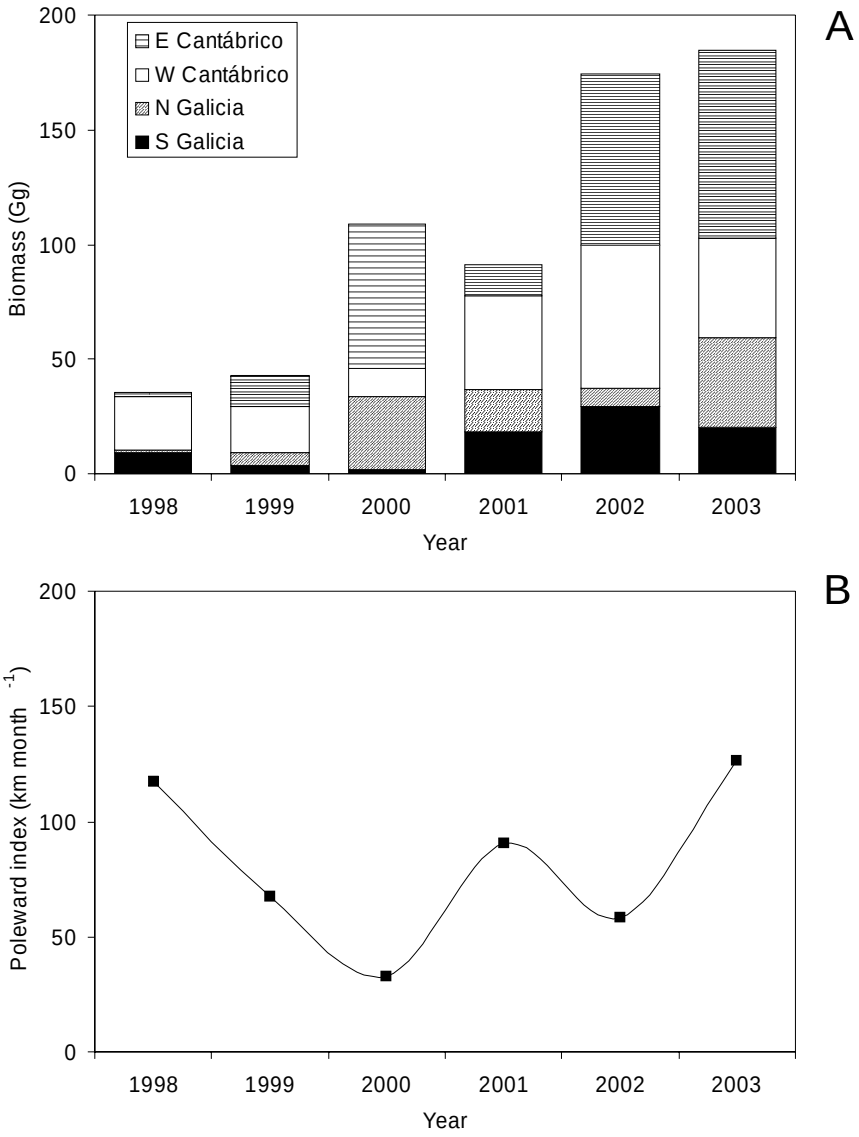

Fig. 5. Interannual variability of sardine biomass (x 10^3 t = Gg) in the study area by zones (A) and interannual variability of the poleward index (km $month^{-1}$) computed from geostrophic winds at 43° N, 11° W between October and December of the previous year (B).